THE EXTERNAL FIBRILLAR SYSTEM OF EUPLOTES WITH NOTES ON THE NEUROMOTOR APPARATUS

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The nature of the external fibrillar system of ciliate Protozoa ("Silberliniensystem" of Klein, 1926*a*, 1926*b*) and its relation to the better-known neuromotor apparatus as first described by Sharp (1914) for *Diplodinium* has been the subject of considerable research of late years. Marked differences of opinion obtain as to the function of both these types of fibers and even their structure has not been agreed upon.

With the hope of solving some of the problems concerned, *Euplotes patella* was chosen for the subject of this investigation chiefly because its neuromotor system is probably better known than that of any other ciliate. Its description given by Yocum (1918) and the demonstration of its function by Taylor (1920) serve as two of the chief milestones toward an understanding of the fibrillar makeup of ciliates.

The description of the external fibrillar network in *E. patella* was originally undertaken by Mr. Samuel Yabroff in 1925 at the University of California. Later he gave up this work when he entered medical school and turned the problem over to me with the statement that he was no longer interested in it.

This work was aided by a research grant from the Graduate School Research Fund of the University of Minnesota.

Technique

Euplotes for this study was cultivated in mass on wheat and timothy hay as described by Turner (1930). The methods of fixation and staining or silver impregnation are outlined below.

For the External Fibrillar System

Silver method of Klein (1926*b*). This gave fair results but drying caused too much distortion. Fixing first in osmic vapor helped some.

Brown's (1930) method of using thionin after Mann's fixation did not show the fibrils at all. Like Liebermann's (1929) nigrosin method it is excellent for *Paramecium* but useless for *Euplotes*.

Silver-gelatin method of Chatton and Lwoff (1930). A few specimens that are around the border of the main drop of gelatin show the network very well, but it is difficult to get just the proper amount of gelatin on the slide.

Formol-osmium-toluidin blue method of Gelei (1927) was useless as no differentiation could be obtained.

The technique which consistently gave the best results for whole preparations was a variation of Klein's and Gelei and Horváth's (1931) methods: Place a small drop of material containing the organisms on a clean slide previously smeared with a light coat of egg albumen. Draw off excess water with a micro-pipette. Fix with osmic acid vapor for about three seconds. This may be easily done by simply inverting the slide over a bottle containing 2 per cent osmic acid. Place slide in a cool breeze (4° to 15° C.) until the material is nearly but not quite dry in the center. Flood gently with two or three drops of 2 per cent silver nitrate. A few of the organisms will be lost but most of them will be caught in the albumen. After 4 to 8 minutes in the silver nitrate, pour off the excess and place the slide in a white dish, or glass dish over white paper, containing enough distilled water to cover the slide. Place in the sun in a cool place and watch the progress of the reduction by occasional examinations with the microscope. When the desired depth has been obtained, wash thoroughly in distilled water, dehydrate, and mount. If slower reduction is desired, exposure to bright sky but not to the sun is efficacious. This method gives strikingly clear-cut results.

Aside from the silver impregnation methods the only way found of demonstrating the network with any degree of clarity and completeness is by drying specimens on a clean slide and staining with thionin. This is done by simply flooding the specimens with a 0.5 per cent aqueous solution of the stain for a few seconds, rinse and dehydrate as rapidly as possible, clear and mount. While this method gives essentially the same picture as the silver methods, less contrast is obtained by it.

Bresslau's (1921) method was used but did not prove satisfactory for *E. patella* although it is good for *Paramecium*.

For sectioning, the only method worth mentioning favorably out of the many tried is that of Gelei and Horváth (1931), using the maximum strength and time for the silver nitrate. After clearing, some of the material was mounted whole and the rest embedded and sectioned 3 to 15 microns thick. These sections were valuable chiefly in showing the location of the fibers in the protoplasm.

Although I have tried many times to repeat the method used by Yabroff (1928) on *Euplotes*, I have consistently failed to get satisfactory results. Others in private correspondence have reported similar failures, but Pickard (1927) reports conspicuous success with it on *Boveria*. In my opinion it is a capricious technique but one which has possibilities and with good fortune and the proper twist of the wrist will be found valuable. *Intra vitam* stains used were neutral red, Janus

green B, dahlia violet, methylene blue, dilute hæmatoxylin, and thionin. All were tried in concentrations of from 1–1,000 to 1–100,000. Neutral red at about 1–10,000 gave the best results.

For the Neuromotor Apparatus

Zenker's and the picro-mercuric fluid of Yocum, followed by Mallory's connective tissue stain as used by Sharp. Sections 2, 4, 6, 8, 10, 15, and 20 microns thick were made in addition to whole mounts. Beautiful preparations were obtained with this stain following either fixative, but the colors fade. In the thicker sections and the whole mounts the general picture is seen fairly well, while the details may be better studied in the thinner sections. Whole mounts were usually fixed in picro-mercuric as the high alcohol-ether content caused the organisms to adhere to the slides much more readily than did Zenker's.

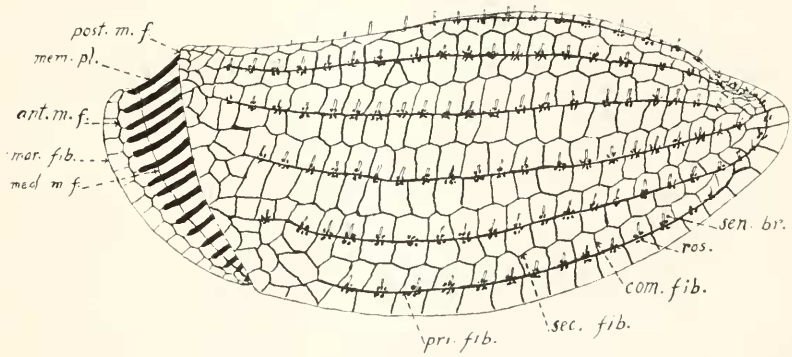


FIG. 1. Dorso-lateral view of external fibrillar system of *Euplotes patella*. Rosettes and sensory bristles from neutral red specimen, the rest from silvered specimen. *Ant. m. f.*, anterior membranelle fibril; *com. fib.*, commissural fibril; *mar. fib.*, marginal fibril: *med. m. f.*, median membranelle fibril; *mem. pl.*, membranelle plate; *post. m. f.*, posterior membranelle fibril; *pri. fib.*, primary fibril; *ros.*, rosette; *sec. fib.*, secondary fibril; *sen. br.*, sensory bristle.

Delafield's, Heidenhain's, Apáthy's and Dobel's hæmatoxylins were used following Schaudinn's, both hot and cold, and Flemming's strong fixing fluids. Schaudinn's followed by Heidenhain's gave the best results with whole mounts while Flemming's and Heidenhain's proved the most satisfactory for sections.

Many other methods were tried without conspicuous success.

Intra vitam stains as mentioned above for the external fibers were used. Neutral red 1–20,000 allowed to act for 1 to 3 hours, and thionin of about the same dilution were found most useful.

The External Fibrillar Network

The convex dorsal surface of *Euplotes patella* shows seven to nine longitudinal rows of granules arranged in little rosettes (Fig. 1). From the center of each rosette a bristle protrudes externally. Griffin (1910) states that in *E. worcesteri* sensory bristles protrude from these rosettes, and recently Jacobson (1931), using a silver technique, describes and figures a central bristle protruding from a ring instead of a rosette, in *E. patella*.

I have observed these bristles not only in material impregnated with silver, but also in living specimens stained *intra vitam* with neutral red. Each bristle is about two microns long and perhaps one-tenth micron

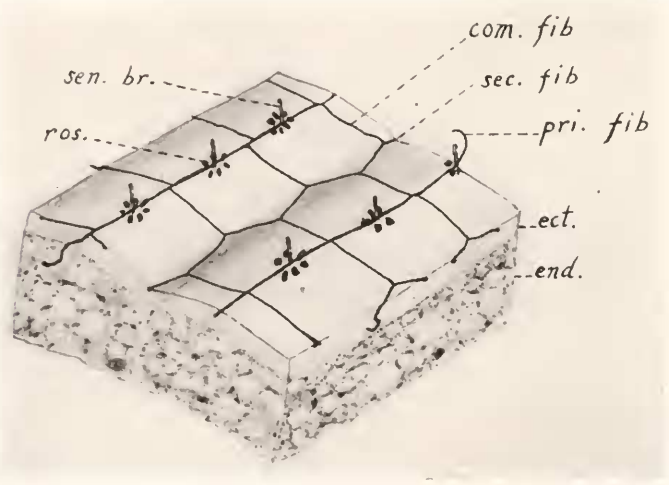


Fig. 2. Enlarged section of dorsal body wall to show position of fibrils in ectoplasm. Note several places where fibrils were not cut off clean with the section. *Ect.*, ectoplasm; *end.*, endoplasm; other labels as in Figure 1.

thick. Its base is surrounded by four to eight elongate granules arranged radially (Fig. 2). In silver preparations the granules of the rosette are usually clumped into a single blob as seen in the photomicrograph (Pls. I, II, and III). That the bristles are not cilia is evident from the fact that they are not vibratile, although it is quite possible they have been evolved from ancestral cilia.

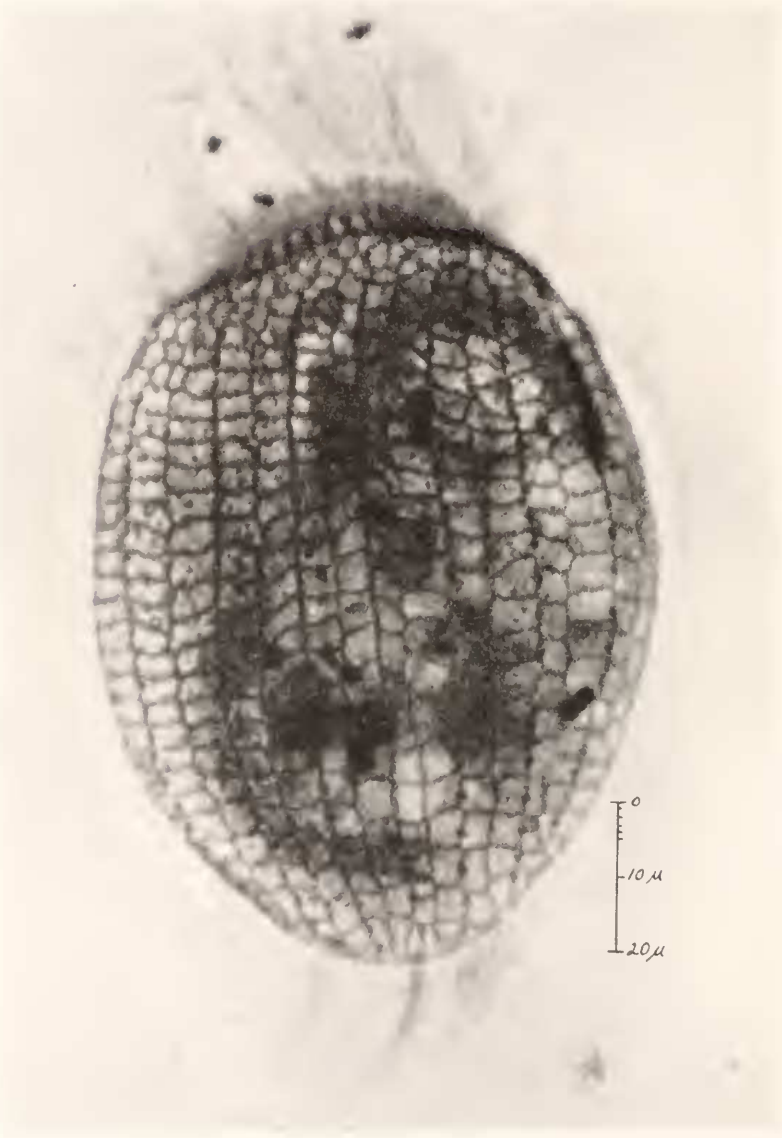
Connecting up the rosettes are seven to nine (usually nine) longitudinal fibrils. These I have called the *primary fibrils* as they are the heaviest and most easily seen, and because they are associated with the bristles and rosettes. Halfway between the primaries and extending parallel to them are the *secondary fibrils* which are only slightly less

regular than the primaries. In addition to these there are *commissural fibrils* extending across from the primaries to the secondaries causing the secondaries to appear as though pulled slightly out of line. This creates a veritable network or latticework which is remarkably constant in appearance, the squares of which average about four microns across (Pls. I and II). All these structures have been observed in neutral red material demobilized with osmic acid vapor. This reminds one of Fig. *F* in Pickard's (1927) paper on *Boveria*. Myonemes, however, have no place in *Euplotes* as the cortex is rigid.

Anterially, the network is connected with the *posterior membranelle fiber* (Fig. 1) at the edge of the dorsal cortex. Both primaries and secondaries are fastened to this fibril either directly or by a more or less irregular anastomosis of the fibrils.

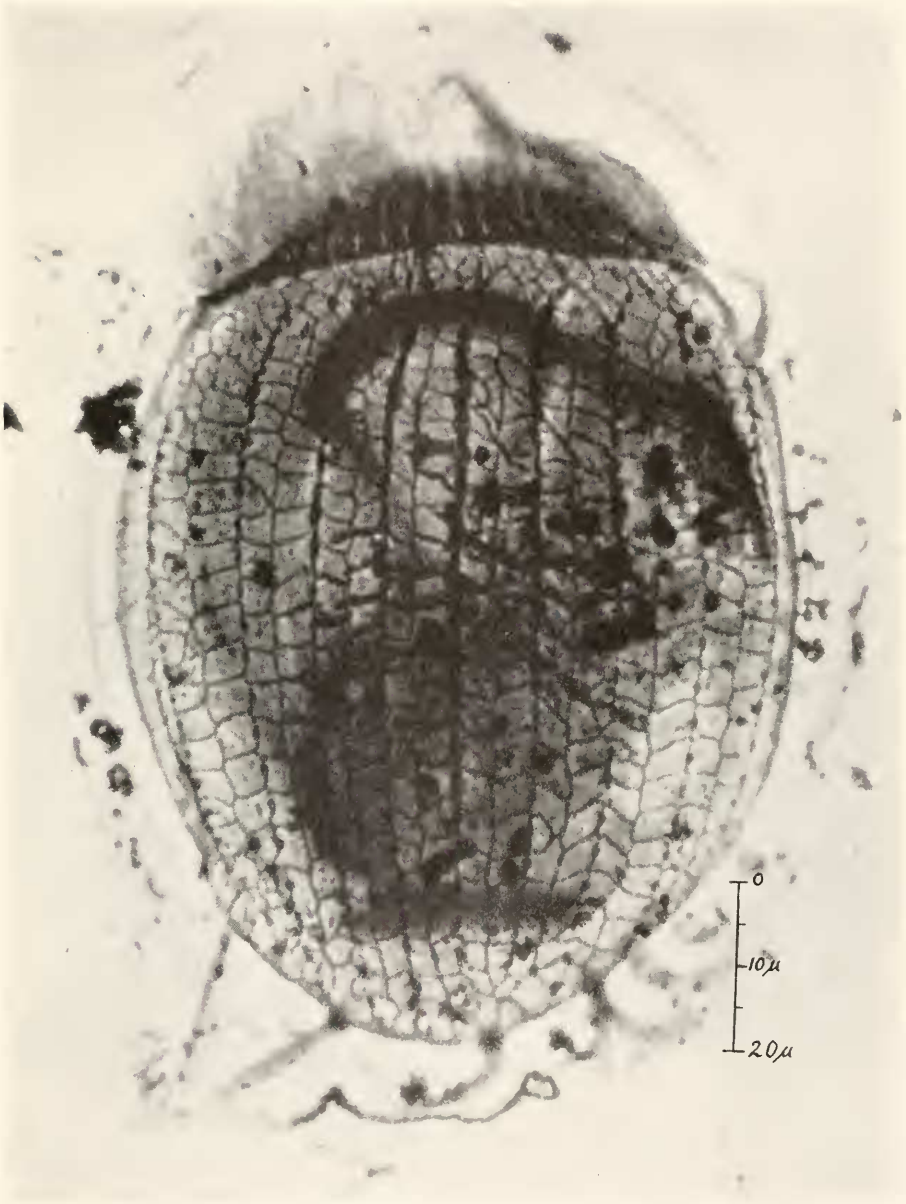
Anterior to the dorsal cuirass is the collar in whose surface is seen an extension of the fibrillar system, part of which was described by Yocum. Extending anterio-ventrally is the row of basal plates of the membranelles (Fig. 1). These are attached to the *posterior membranelle fibril* at the proximal end and extend about two-thirds the distance to the margin of the lip where they connect with the *anterior membranelle fibril* by means of short commissures that extend from their tips. This anterior membranelle fibril is the "membranelle fiber" (anterior cytostomal fiber) of Yocum's neuromotor apparatus. This in turn is connected to the marginal fibril by short commissures. Linking up the basal plates of the membranelles is still another fibril about two-thirds the distance to their anterior tips. This *median membranelle fibril* is seen only in the clearest preparations as it is easily blocked out by the heavy impregnation of the membranelle plates. In all probability it was part of this collar equipment that Yocum saw and considered sensory structures.

On the ventral surface of *E. patella* the network, instead of being composed of a cross-hatching of lines, appears less regular and reminds one of badly treated chicken wire (Plate III). Hexagons form a rather prominent part of the network while rectangles, squares, etc., are not infrequent. The pattern, however, as seen in any of the individuals is surprisingly constant and characteristic. For example, the hexagons of the ventral surface of the oral lip, the long slender rectangles extending posterior to the tip of the peristomal field, and the squares of the lateral phlanges are always present. The squares of the lateral phlanges are made by fibrils similar to those of the dorsal surface, the marginal fiber being a secondary and the submarginal being a primary fibril provided with rosettes and sensory bristles as are those of the dorsal surface.



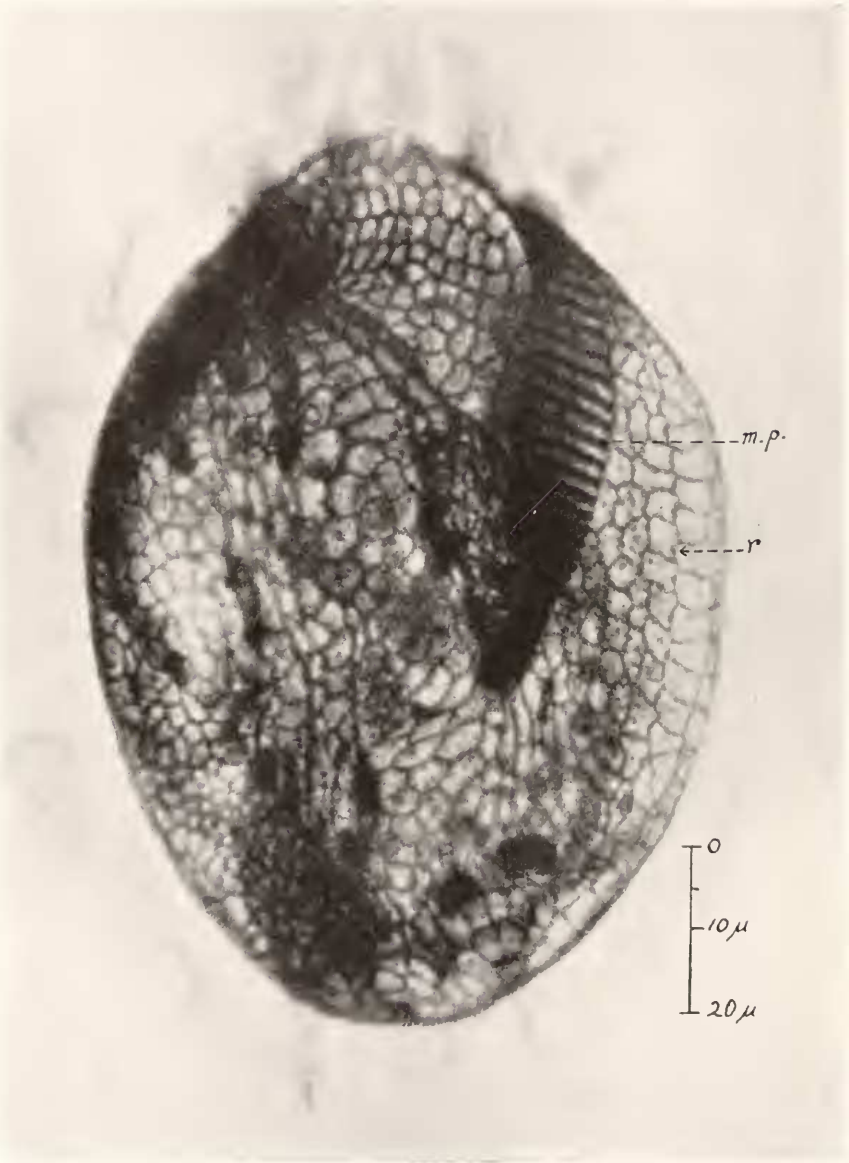
EXPLANATION OF PLATE I

Photomicrograph of dorsal network of *E. patella*. Silver nitrate preparation.



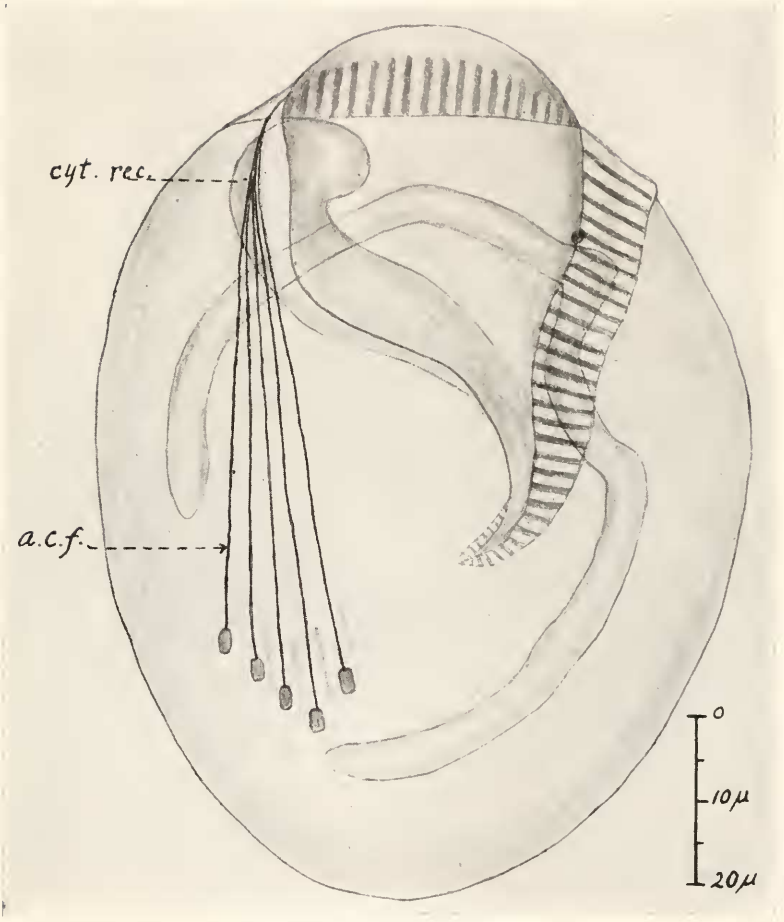
EXPLANATION OF PLATE II

Photomicrograph of dorsal network of another specimen. Rosettes can be seen clearly near the sides as single blobs. Silver nitrate preparation.



EXPLANATION OF PLATE III

Photomicrograph of ventral network of *E. patella*. *m. p.*, membranelle plate in lateral peristomal field; *r.*, rosette. Silver nitrate preparation.



EXPLANATION OF PLATE IV

Camera lucida drawing, ventral view, of *E. patella* showing the anal cirri fibers of the neuromotor apparatus passing directly into the collar, unbroken by a motorium. All other cirri and fibers omitted from the drawing. *A. c. f.*, anal cirri fibers; *cyt. rec.*, cytostomal recess. Schaudinn's and Heidenhain's hæmatoxylin.

The basal plates of the membranelles located in the left margin of the peristome (Plate III and Fig. 3) are deeply impregnated by the silver and appear like ties of a railroad track. Extending down the center of the "roadbed" like a loosely strung wire is the fibril which connects basal plate to basal plate—a continuation of the median membranelle fibril. Bordering each side of the peristomal field of membranelles and in direct contact with the ends of the basal plates are the two other membranelle fibrils.

Sectioned material shows the fibrils of both dorsal and ventral networks to be immediately under the pellicle and in contact with it. They appear round in cross-section and the fibrous nature is disclosed by the fact that frequently they are not cut off clean with the section but protrude from the edge like loose threads at the end of a frayed piece of cloth (Fig. 2).

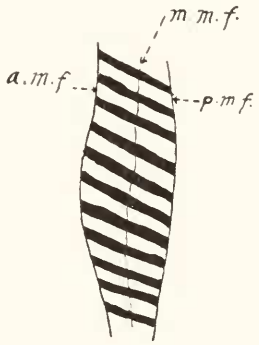


FIG. 3. Camera lucida sketch of a portion of the membranelle field at about point *m. p.* in Plate III, but taken from another specimen. *A. m. f.*, anterior membranelle fibril; *m. m. f.*, median membranelle fibril; *p. m. f.*, posterior membranelle fibril, all continued down from collar region. Silver nitrate preparation.

It is worthy of note that the apparent heavy nature of the fibrils is due to the impregnation of silver, as in material stained *intra vitam* the fibrils appear as exceedingly delicate strands.

NEUROMOTOR APPARATUS

Regarding the neuromotor apparatus, my observations tend to confirm those of Yocum except in the matter of the motorium. The fibers from the bases of all cirri except the anals were found to extend considerably farther through the cytoplasm than indicated by Yocum, but this is a matter of no special significance.

After examining literally hundreds of specimens both entire and in serial sections 2, 3, 4, 6, 8, 10, 15 and 20 microns thick, using all the

techniques suggested for its demonstration, I am forced to the conclusion that there is no motorium in *Euplotes patella* as a separate and distinct body. I have never been able to see it even in specimens where the other neuromotor elements are exceptionally clear. It is quite evident in many of my preparations, both whole mount and sectioned, that the five anal cirri fibers converge, join to form one fiber and extend on into the collar without a break (Plate IV). Here the single fiber turns and runs along the anterior (outer) edge of the "collar" membranelles thence along the inner (median) edge of the "lapel" membranelles. Yocum shows the five fibers attached to the left end of the motorium (his Plate XV, Fig. 9) while the fiber to the membranelles is attached to the right end of the motorium. This makes quite a break in the continuity of the system. It is easily seen in my material that there is absolutely no jog or break in the fibers from the time they leave the cirri until they reach the membranelles. Furthermore, there is no branch line running off to any body that might be considered an attached motorium, nor is there a body which is nearby and consistently present which might be a motorium.

It is quite possible that any one of several structures in this region might be mistaken for a motorium, namely, the basal plate of one of the two or three cirri located there, groups of granules such as are found scattered through the cytoplasm, or a fold of the ectoplasm in the rather thin right peristomal phlange. Professor Yocum has kindly sent me one of his slides showing what he considers the motorium on two marked specimens. Although it is hardly just to judge from two specimens, it seems to me that the darkened area which he interprets as the motorium is a wrinkle in the ectoplasm overlying the fibers on the ventral surface. I find similar structures in my own preparations if the material is fixed on slides but not in material killed in bulk. The logical explanation of this is seen in the anatomy of the organism. In the region concerned the roof of the cytostome contains a peculiar pocket, the cytostomal recess (Plate IV). When the animal is flattened by being fixed on the slide the peristomal phlange in this region is folded by being pressed against this irregularity. Such a fold occurring regularly at this point could easily be interpreted as a motorium. Yocum's Figure 9, Plate XV, can be explained in this light if the part of the membranelle fiber before it reaches the collar be considered a continuation of the edge of the cytostomal recess. However, his Fig. 5, Plate XIV, is difficult to explain on these grounds. Such a structure might have resulted from a collection of endoplasmic granules as so frequently occurs in this species. My interpretations must be based on the fact that in my material no such body is present as that which he labels "motorium,"

and the membranelle fiber, instead of giving off two roots to this body, is clearly and directly continuous with the five anal cirri fibers. Serial sections stained with Mallory's triple and with the hæmatoxylins support the camera lucida drawing of the whole mount shown in Plate IV.

Rees (1930) concludes that in *Diplodinium* the object called a motorium by Sharp (1914) is only a fold of the ectoplasm, and the careful investigation that he made is quite convincing. The "motorium" of *Euplotes patella* is probably a similar structure.

Discussion

The composite picture of *Euplotes patella* shows an amazing array of fibrils which link up the organelles. This consists of a superficial network linking up bristles that protrude from the surface, and a deeper set which is associated with the motor organelles. The two are connected in the anterior region.

This picture in itself strongly suggests a sensory apparatus and a motor apparatus linked together to form an integrated coördinating system.

Klein (1926*a*) was the first to give us a detailed picture of the external fibrillar network in a ciliate. Since then he has described the "Silberliniensystem" of a number of ciliates, some of which are beautifully illustrated, some very poorly. The latter includes a distorted and almost unrecognizable *Euplotes* of undetermined species (Klein, 1928). In this paper he states that the new peristomal field (as seen in the new complex of fibers) appears before any other sign of division. Turner (1930) has shown, however, that the macronucleus is the first structure to show signs of an approaching division. In another paper Klein (1926*b*) pictures the rosettes of *E. harpa* as single granules and calls them "Basalkörner." In this paper he proposes the idea that the fibrillar network is the real coördinating system in ciliates, and in *Euplotes* it is the real neuromotor system while the system described by Yocum is contractile in function. According to him the microdissection experiments of Taylor were confused. Claiming it to be a primitive nervous system endowed with both motor and sensory functions, Klein (1929) further ascribes to the "Silberliniensystem" the power of initiating division, controlling morphogenesis and to some extent inheritance. His evidence for all this is not completely convincing.

The German workers generally consider the "classical" neuromotor apparatus as a contractile or supportive structure rather than conductile. In *E. patella* the only possible function of contractile elements would be for the operation of the cirri or membranelles, and as these motor organelles are actually groups of cilia, one would not expect them to be

operated by such contractile fibers on the basis of what is known of ciliary movement. Taylor's observations show also that the anal cirri continue to function even after the fibers are cut, a fact that rather demolishes the notion that the contraction of the fibers mechanically operates the cirri. Again, the fact that no movement can be detected in the fibers when the cirri are beating is further evidence against this idea.

Without a motorium the neuromotor system of *E. patella* is still established as a definite apparatus. A coördinating *center* is a nice concept, but one which is not indispensible to a neuromotor apparatus. Taylor's results are as significant without a motorium as with, when one considers the fibers themselves as the coördinating mechanism; the continuity of the fibers from the membranelles to the anal cirri maintaining the coördination.

The function of the external fibrillar network is less well established. In *E. patella* where the cortex is so strong and unyielding it is difficult to believe that a network so delicate as that seen in living specimens could add much to the rigidity of the body. From purely morphological evidence it appears that the network is sensory in nature. It is just under the pellicle where one would expect such a system, and it connects up all the bristles which appear to be sensory elements. The joining of this system to the neuromotor fibers makes a complete conductor system—sensory and motor—that seems not only adequate to interpret the structures seen, but also to explain what is known of the reactions of the organism.

In the more primitive ciliates with cilia distributed over the body, the "silver lines" may well be both sensory and motor in function, forming a kind of primitive coördinating apparatus which controls the action of the cilia in response to stimuli received. In *Euplotes,* which is one of the most highly organized of all the Protozoa, it is not surprising to find the conductor system more or less divided up into sensory and motor departments comparable in a way to the more highly specialized members of the Metazoa.

Summary

1. The external fibrillar system or "Silberliniensystem" of *Euplotes patella* is described as a regular latticework on the dorsal surface and a more irregular network ventrally.

2. These lines are associated with rows of rosettes from which bristles protrude on both dorsal and ventral surfaces. These bristles are thought to be sensory in function and the network a sensory conductor system.

3. The neuromotor system was studied and Yocum's description supported except for the motorium. Evidence is presented which seems to indicate that there is no motorium in this species.

4. The external network is directly connected with the neuromotor system.

5. Discussion brings out the probability that the neuromotor apparatus in *E. patella* is thus augmented by a distinct but connected external network of sensory fibrils.

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